GROWTH AND REGENERATION RATES OF THE CALCAREOUS SKELETON OF THE CARIBBEAN CORALLINE SPONGE *CERATOPORELLA NICHOLSONI*: A LONG TERM SURVEY

PHILIPPE WILLENZ AND W.D. HARTMAN

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The growth rate of the aragonitic skeleton of the Caribbean 'sclerosponge' *Ceratoporella nicholsoni* has been studied by *in situ* staining of specimens with calcein in a reef tunnel, 28m depth, near Discovery Bay, Jamaica. Experiments were performed up to five times from 1984 to 1997 on a population of 10 specimens ranging from 10-20cm maximum diameter. In each experiment small skeletal samples were removed from the periphery of sponges, and specimens were left in place for further studies on growth and regeneration. Perpendicular sections, ground to a thickness of about 10µm, were photographed by fluorescence microscopy. Annual skeletal growth rates were calculated from measurements of the linear extension between calcein stained lines along growth axes. Data indicate that although average annual growth rates remained in the same range for different periods (214.6±54.5-233.3±33.0µm yr^{-1}), significant differences occurred from one individual to another within the same period. The annual growth rate of a given individual also varied significantly in time (191.1±30.0-269.9±37.0µm yr^{-1}). A second population of smaller individuals, measured after a single period of one year, revealed a strikingly lower average annual growth rate (124.4±35.0µm yr^{-1}). Regeneration of the skeleton of injured specimens was also characterised by an initial slower growth rate. Nevertheless, after the first year, it was comparable to normal growth, and exceeded it slightly thereafter. This first long term study of *Ceratoporella* provides important information on the variability in growth rates, with implications on the use of selerosponges as paleoenvironmental proxies. ❐ *Porifera, sclerosponges, coralline sponges, growth rate, aragonite, skeleton, regeneration, calcein, Ceratoporella nicholsoni.*

Philippe Willenz (email: pwillenz@ulb.ac.be), Department of Invertebrates, Royal Belgian Institute of Natural Sciences, 29 Rue Vautier, B-1000 Brussels, Belgium; W.D. Hartman, Yale University, Peabody Museum of Natural History, P.O. Box 208118, New Haven, CT, USA 06520-8118; *18 January 1999.*

Although the first specimen of the coralline sponge *Ceratoporella nicholsoni* was dredged off Cuba in 1878, and described as a new alcyonarian coelenterate more than thirty years later (Hickson, 1911), it was not until the mid-1960s that this species was rediscovered (Hartman & Goreau, 1966), and subsequently shown to be a sponge (Hartman & Goreau, 1970). At the same time, thanks to the increased use of SCUBA diving, the extent of the diversity of 'sclerosponges' became evident (Hartman, 1969; Hartman & Goreau, 1970, 1975).

Among the nine known species of Caribbean coralline sponges, *Ceratoporella nicholsoni* secretes the most massive basal skeleton of calcium carbonate. Despite the fact that the ecology and ultrastructure of *Ceratoporella* have now been extensively investigated (Lang et al., 1975; Willenz & Hartman, 1989), the growth rate of its aragonitic skeleton is still unknown, more than a century after its discovery.

Both direct staining and indirect techniques have been used to evaluate the growth rate of *Ceratoporella*: the former using alizarin red (Dustan & Sacco, 1983) or calcein stains (Willenz & Hartman, 1985); the latter based on ^{14}C and ^{210}Pb chronologies (Benavides & Druffel, 1986; Druffel & Benavides, 1986) or focusing on carbon and oxygen isotope studies (Joachimski et al., 1995; Böhm et al., 1996). Considering the estimated slow calcification rate of this species, the latter techniques are the most convenient for elucidating long term information (time scales of tens of years to centuries). Direct methods, however, have the potential advantage of revealing data on growth rates for shorter time scales (years to decades).

Calcein was first used in invertebrates to mark

TABLE 1. *Ceratoporella nicholsoni.* Successive *in situ* labeling with calcein (*). Abbreviations: T1, 9.VII.1984; T3, 15.II.1985; T4, 29.IV.1986; T5, 1.V.1987; T6, 1.V.1997.

Specimen number	Experimental period				
	T1	T3	T4	T5	T6
4	*	*	*	*	*
7	*	*	*	*	*
8	*	*	*	*	
9		*	*	*	*
10		*	*	*	*
11		*	*	*	*
14		*	*	*	*
16	*	*	*	*	*
17			*	*	*
27				*	*
29				*	*
DURATION					
T1-T3	221 days				
T3-T4		438 days			
T4-T5			363 days		
T5-T6				10 years	

the newly deposited calcium carbonate of the basal skeleton of *Ceratoporella* (Willenz & Hartman, 1985). Subsequently, this chemical has been used to record calcification amongst a wide variety of taxa such as brachiopods, bryozoans, molluscs and echinoderms (reviewed in Rowley & Mackinnon, 1995). More recently it has also been employed in studies of the growth dynamics of calcareous sponge spicules (Ilan et al., 1996), or to estimate the growth rate of the Indo-Pacific coralline sponges *Acanthochaetetes wellsi* (Reitner & Gautret, 1996) and *Astrosclera willeyana* (Wörheide, 1998).

From these studies, calcein appears to be permanently bound to calcium carbonate that forms in the presence of the dye, although the chemistry of the process has yet to be studied. Calcein has the advantages of fluorescing brightly under UV light and having only weak toxicity.

Several specimens of *Ceratoporella nicholsoni*, including the four individuals used in the first experiment by Willenz & Hartman (1985), were repeatedly stained and sampled at different intervals over 13 years, in order to evaluate potential growth rate variations among the sponges during extended periods of time.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN. Two size categories of *Ceratoporella nicholsoni* were studied in a reef tunnel at depths ranging from 25-29m at Pear Tree Bottom, 5km E of Discovery Bay, Jamaica. The largest individuals, 10-15cm diameter, were labelled with calcein (Fluka 21030) *in situ* without being removed from their substrate. Labelling was performed from 1984 to 1997 at intervals given in Table 1. After the initial labelling in July 1984 (T1) a second incubation was performed six days later (T2), to test the

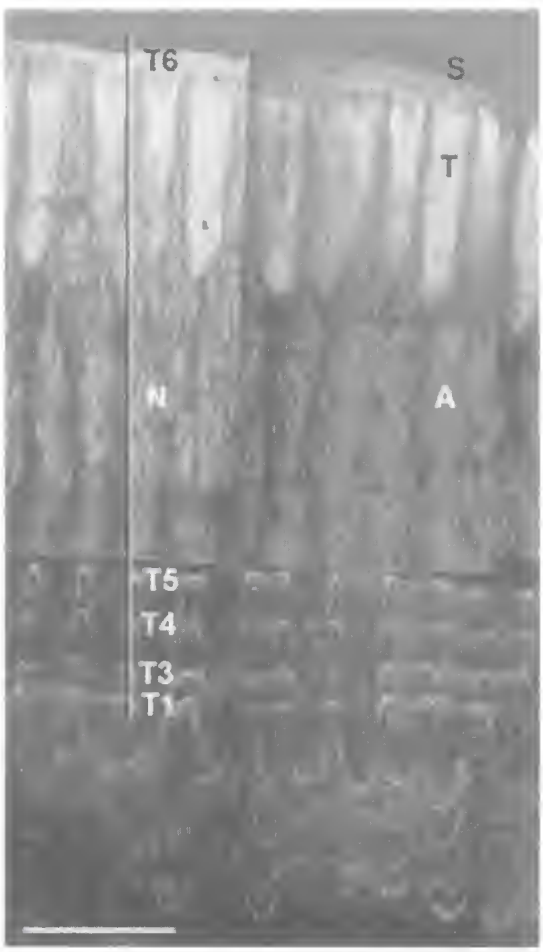

FIG. 1. *Ceratoporella nicholsoni.* Natural growth pattern. Ground section from specimen no. 16 sampled in May 1997, viewed by epifluorescence microscopy. Successive labeling events with calcein are indicated at apex of walls separating pseudocalicles, along aragonitic skeleton (A) extension axis. Living tissue (T) is brightly fluorescent. N=natural growth axis, S=surface of living tissue. (Scale bar=500µm).

TABLE 2. *Ceratoporella nicholsoni*. Annual growth rates during the 10 year experimented period. A, Kruskal-Wallis ANOVA on ranks (H=379.5 with 9 degrees of freedom; P<0.0001). B, All pairwise multiple comparison procedures (Dunn's Method). NS indicates no significant difference and * indicates significant (P<0.05) difference. Both statistics indicate a significant variability between specimens (P<0.005).

A. Specimen	Median	25%	75%	Mean	N
4	236.0	228.0	242.0	234.6	42
4	224.0	221.5	228.0	224.3	29
4	280.0	275.5	284.0	279.4	33
10	219.0	210.0	224.0	216.9	36
10	248.0	242.0	254.5	249.5	37
14	290.0	281.5	296.0	287.3	61
16	232.0	228.0	242.0	234.9	85
17	215.0	212.0	222.0	215.8	18
27	217.1	214.7	219.6	216.5	28
29	173.7	170.4	178.2	173.6	59

B. Specimen	4	7	9	10	11	14	16	17	27	29
4	-									
7	NS	-								
9	*	*	-							
10	*	NS	*	-						
10	NS	*	NS	*	-					
14	*	*	NS	*	*	-				
16	NS	NS	*	*	NS	*	-			
17	*	NS	*	NS	*	*	*	-		
27	*	NS	*	NS	*	*	*	NS	-	
29	*	*	*	*	*	*	*	NS	NS	-

sensitivity of the method. Although distinct bands could be detected at a distance of about 4μm (Willenz & Hartman, 1985), interval T1-2 was omitted in this analysis because of the shortness of the time period involved. Additional smaller specimens (11-25mm diameter) were removed from the substrate and cemented *in situ* to Plexiglas plates (5 specimens /12x12cm plate) with epoxy underwater patching compound (Pettit Paint Co no. 7050 & 7055). Plates were stored in Plexiglas racks placed on a ledge of the tunnel at the depth of collection.

To label the sponges with dye, the large specimens were individually enclosed within a plastic bag (of 4L volume) that was secured around the base of the sponge with nylon cords or rubber bands. In the case of plates bearing small specimens, the Plexiglas racks securing the plates were enclosed in a plastic bag. Calcein, dissolved in sea water, was injected in each bag to reach a concentration of 100mg/l. Bags were removed from the sponges after 12 or 24hrs.

For large specimens, samples of the skeleton, with attached living tissue, about 1-3cm^3 in volume, were removed with hammer and cold chisel from the periphery of the sponge, each specimen was left in place for further growth and regeneration. Small specimens were sacrificed after one year. Following dehydration in a graded series of alcohols, samples were embedded in Spurr's medium (Spurr, 1969). Sections, cut with a low speed diamond saw (Bennet Labcut 1010) were mechanically ground on a series of diamond grinding disks (Buehler ultra-prepTM) using a semiautomatic grinder (Buehler Minimet 1000) to a thickness of 5-10μm and observed under epifluorescence microscopy (Nikon Optiphot-2 microscope, excitation filter 340-380nm, barrier filter 420nm).

Growth increments of the aragonitic skeleton were established by measuring the linear extensions (in micrometers) between stained lines along growth axes at the apical edges of the wall separating two pseudocalices, or, for the most recent period, between stained lines and the surface of the skeleton.

DATA ANALYSIS. Statistical analyses were performed using SIGMASTAT and SIGMAPLOT (Jandel Scientific) data analysis and graphics software. All linear extension measurements were normalised as annual growth rate prior to their analysis. Non parametric Kruskal-Wallis analysis of variance (ANOVA) on ranks was performed to test two null hypotheses. 1) H_0: there are no differences in the average growth rates among specimens of *Ceratoporella* within a given

TABLE 3. *Ceratoporella nicholsoni*. Comparison between linear annual growth rate and regeneration growth rate. Unavailable data due to bioerosion in a specimen are indicated (*).

Period	Linear growth rate	Regeneration growth rate	Specimens
T3-4	230.5 + 61.2	194.2 + 42.2	7 - 9 - 10 - 17*
T4-5	232.0 + 59.6	238.4 + 38.8	7 - 9 - 10 - 11 - 17
T5-6	244.4 + 25.10	272.2 + 36.9	9 - 10 - 11 - 16

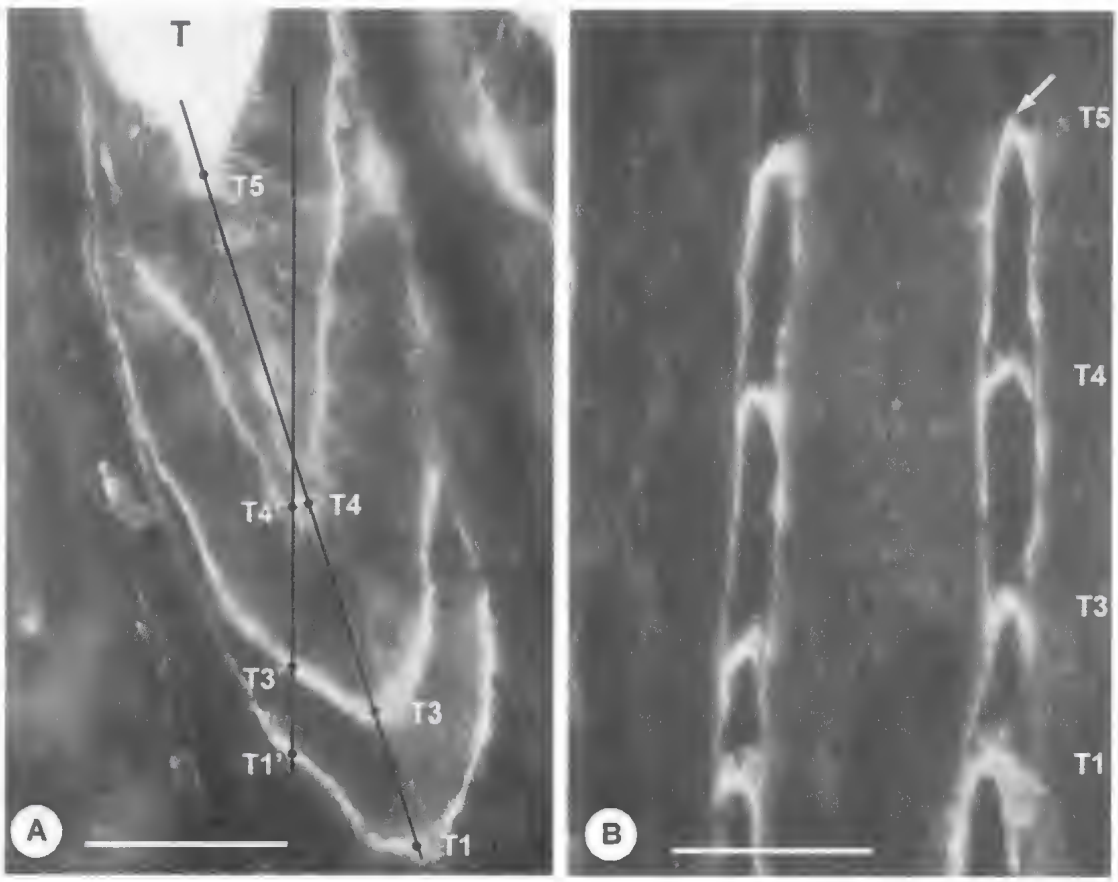

FIG. 2. *Ceratoporella nicholsoni*. A, ground section of fragment sampled at T5, view at base of pseudocalyx. Two possible orientations of section plane indicate that measurement can easily be biased when section is not parallel to growth axis (T1'-T3' < T1-T3; T3'-T4' < T3-T4). T= living tissue. (Scale bar=250μm); B, ground section of fragment sampled at T6, view at apex of wall separating two pseudocalices. Narrow structure of walls (arrow) prevents reading errors. Here, space between two walls has been filled as sponge grew upward. (Scale bar=250μm).

TABLE 4. *Ceratoporella nicholsoni*. Measurement reproducibility test. A Mann-Whitney test indicates that differences obtained from two different slides of the same sample are not significantly different (P < 0.005), except for specimen 16 in period T3-4.

Specimen slide no.	Period T3-4			Period T4-5		
	Mean	n	P	Mean	n	P
7 a	273.4	24	0.1120	287.6	24	0.0395
7 b	260.9	20		266.6	21	
9 a	268.1	35	0.0272	254.3	35	0.6680
9b	258.5	35		254.8	35	
10 a	160.7	26	0.7190	204.8	26	0.7490
10 b	159.1	24		202.4	24	
16 a	177.0	35	0.0054	211.6	35	0.7960
16 b	189.7	35		211.2	35	

period; 2) H_0: there are no differences in the average growth rates among various periods for a given specimen (Sokal & Rohlf, 1981; Fox et al., 1994). Where the ANOVA on ranks rejected the null hypothesis the Dunn's all pairwise multiple comparison procedure was used to determine the groups that differed from each other (P<0.005 level). A Mann-Whitney rank sum test was used where only two groups were to be compared. Significant differences were concluded at P<0.005 level. Data reproducibility was tested for four of the largest specimens by comparing measurements from a second ground section prepared from the same fragment.

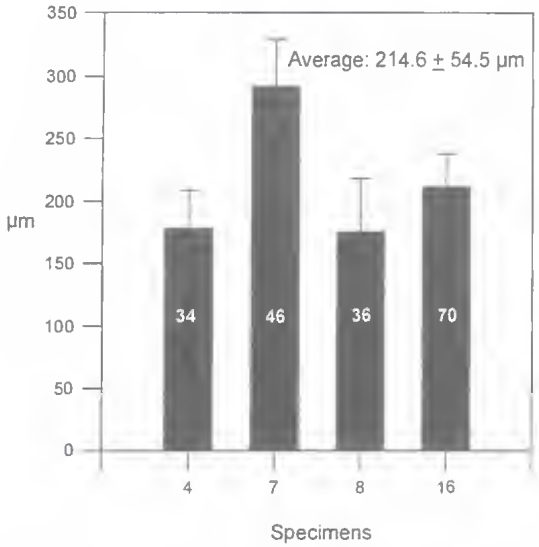

FIG. 3. *Ceratoporella nicholsoni*. Mean linear annual growth rates (µm yr $^{-1}$) of 4 specimens during period T1-3 (221 days). Average of all specimens is indicated. Numbers of measurements are indicated within bars. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except between specimen numbers 4 vs 8.

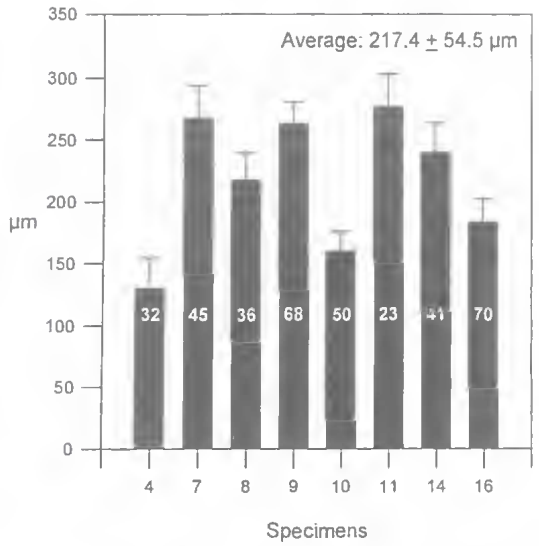

FIG. 4. *Ceratoporella nicholsoni*. Linear annual growth rates (µm yr $^{-1}$) of 8 specimens during period T3-4 (438 days). Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except between specimens 11 vs 14, 14 vs 16, 8 vs 16, 16 vs 10, and 10 vs 4.

RESULTS

LINEAR ANNUAL GROWTH RATES.

Observation of ground sections of fragments of *Ceratoporella nicholsoni* in epifluorescent microscopy revealed successive labelling with calcein (Fig. 1). Figure 2A-B shows details of the bright fluorescent bands at the base of a pseudocalicle and at the apex of walls separating two units, respeetively. It is shown that variations in the orientation of sections can induce larger measurements errors at the base than at the apex. Consequently, only measurement at the apexes were considered.

Figure 3 presents the means of measurements of the four specimens of *Ceratoporella* successively marked during the first period T1-3 (221 days), as well as the average growth rate of the population. Thc rcsults of Kruskal-Wallis ANOVA on ranks test indicate signifieant variability between the means ($P<0.0001$). However, a Dunn's all pairwise multiple comparison procedure determined that specimens no. 4 and 8 did not differ from each other.

Figures 4-6 present the same procedure for periods T3-4 (438 days), T4-5 (363 days) and T5-6 (10yrs), respectively. Identical statistieal tests also indicate a significant variability between specimens, except for pairs indicated in the captions. In period T4-5, a batch of smaller samples gave rise to an average annual value (124.4±35.0µm yr^{-1}) reaching only half the average annual growth rate of the larger specimens (233.3±45.0µm yr^{-1}). However, individual measurements did not reveal a direct correlation between the size of sponges and their growth rate.

Table 2 presents the results of a Kruskal-Wallis ANOVA on ranks on data available from the longest interval (T5-6), showing a significant variability between specimens ($P<0.005$). An all pairwise multiple comparison procedure indicates in detail which pairs are significantly different.

Comparison of the mean annual growth rates of specimens from one period to the other (Fig. 7) also indicates a significant variability (191.1±30-269.9±37.0µm yr^{-1}), revealing that individual growth rate amongst *Ceratoporella* was not steady either.

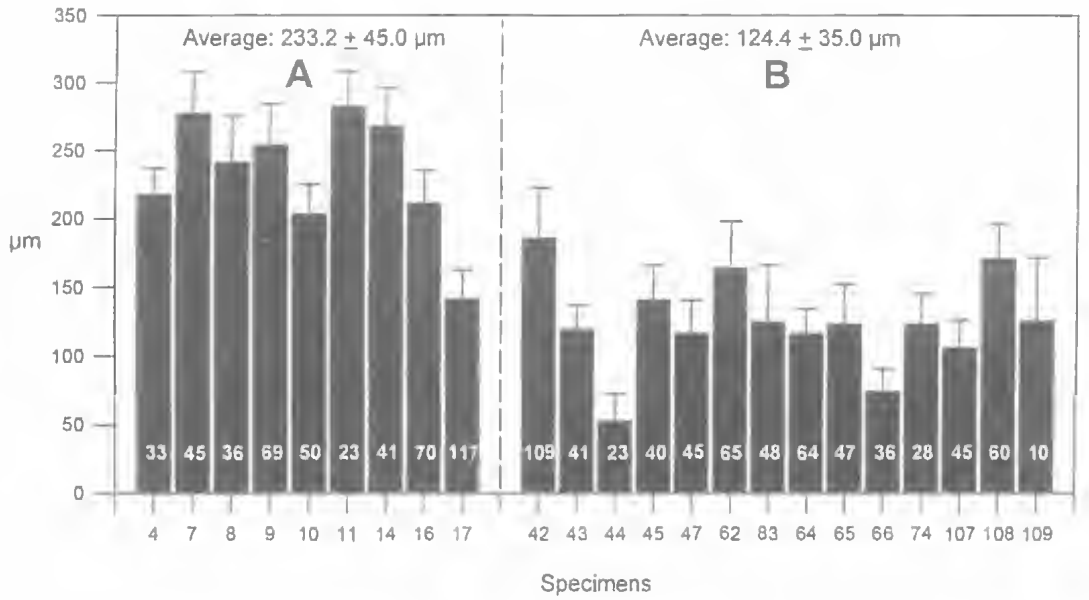

FIG. 5. *Ceratoporella nicholsoni*. Linear annual growth rates (µm yr^{-1}) of A, 9 large specimens and B, 14 smaller ones during period T4-5 (363 days). Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except between specimens 4 vs 10, 8 vs 11, 8 vs 16, for the large specimens and specimens 42 vs 62, 45 vs 64, 108 vs 109, 109 vs 107, 107 vs 44 for the small ones. Both populations have distinct average annual growth rates.

REGENERATIVE ANNUAL GROWTH RATES. Each sampling caused an injury to the sponge, leaving a bare fracture of the skeleton (Fig. 8A). The living tissue rapidly extended over this fracture within a few weeks and covered it completely after a month (observations reported by a local diver). No detailed measurement was done, but at most, after 200 days the naked fracture was healed (shortest interval between personal observations).

Subsequent labelling followed by re-sampling in the healed zone (Fig. 8B) provided direct observation on the regeneration pattern of the skeleton. Ground sections show that new walls are progressively erected to form new pseudocalices perpendicularly oriented toward the fracture (Fig. 9).

Measurements of the extension of the skeleton show that in the first period following an injury, the average regeneration rate is lower than the average normal linear growth rate measured on the same specimens (Fig. 10, Table 3). In the subsequent periods, the regeneration rate increased, exceeding progressively the normal average growth rate.

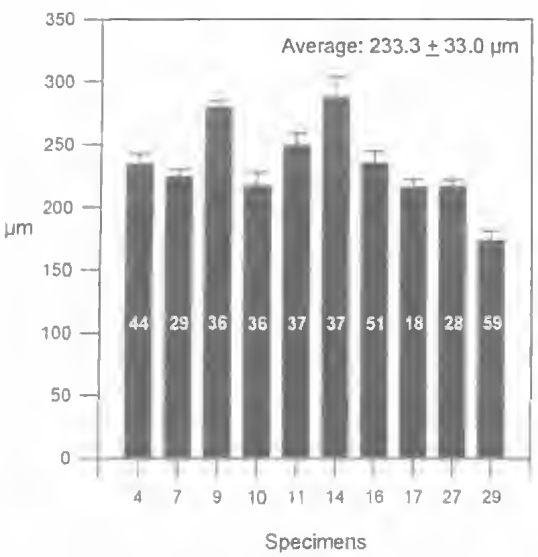

FIG. 6. *Ceratoporella nicholsoni*. Linear annual growth rates (µm yr^{-1}) of 10 specimens during period T5-6 (10 yr). Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between specimens ($P<0.005$), except as indicated in Table 2.

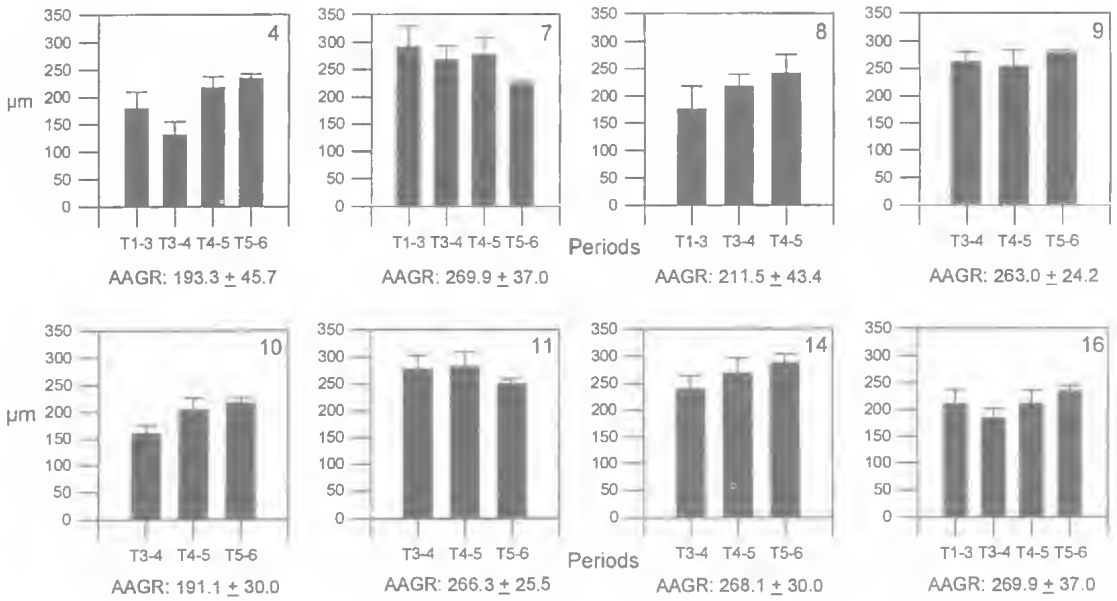

Fig. 7. *Ceratoporella nicholsoni*. Growth rate variability within samples, from one experimental period to another. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant variability between means ($P<0.005$), except for the following specimens and periods: specimen 4 (T4-5 vs T5-6), specimen 7 (T1-3 vs T4-5; T 3-4 vs T4-5), specimen 10 (T4-5 vs T5-6), specimen 11 (T3-4 vs T 4-5), specimen 16 (T1-3 vs T4-5). Specimen numbers are indicated within each graph. AAGR= Average annual growth rate.

Obvious but unexpected marks from sampling were noticed on several specimens when revisiting them in May 1997 (Figs 8A-8B). Measurement of the extension of those particular regeneration areas indicates that someone had shown interest in our samples exactly one year before. Luckily, only one specimen had disappeared (specimen no. 8).

REPRODUCIBILITY OF THE MEASUREMENTS. In order to test the reliability of the method, measurements of pairs of sections prepared from the same fragment for four of the largest specimens were compared. A Mann-Whitney test indicates that differences obtained from two different slides were not significant, except for one specimen (Table 4).

DISCUSSION

Both tetracycline and calcein were used to initiate this study (Willenz & Hartman, 1985). First experiments found that tetracycline failed to label the skeleton of *Ceratoporella*. Although no harm was apparent to the organism, further experimentation in an attempt to adjust the concentration of the dye, to improve its recovery in the skeleton, was abandoned. This was decided in consideration of the potential effects the antibiotic might have on the abundant intercellular symbiotic bacteria present in sponge tissues (Willenz & Hartman, 1989; Hartman & Willenz, 1990). Calcein, first used then on invertebrates, was considered as a more appropriate benchmark to measure 'growth since marking'. At that time, there was no indication of the permanence of its strong fluorescence for long term measurements, whereas this study clearly showed that calcein is stable enough to mark aragonite for at least 13 years.

Based on calcein-labelling experiments, the average annual growth rates of *Ceratoporella nicholsoni* were shown to remain in the same range throughout the different experimental periods (214.6±54.5-233.3±33.0μm yr^{-1}). Measurements made over the largest time interval (10yrs) are obviously most indicative of average annual growth rates (233.3±33.0μm yr^{-1}). These values are comparable to other studies based either on indirect radiometric methods [270μm yr^{-1} using ^{14}C, and 220μm yr^{-1} using ^{210}Pb (Benavides & Druffel, 1986; Druffel & Benavides, 1986), 180-260μm yr^{-1} (Joachimski et al., 1995), and 220μm yr^{-1}

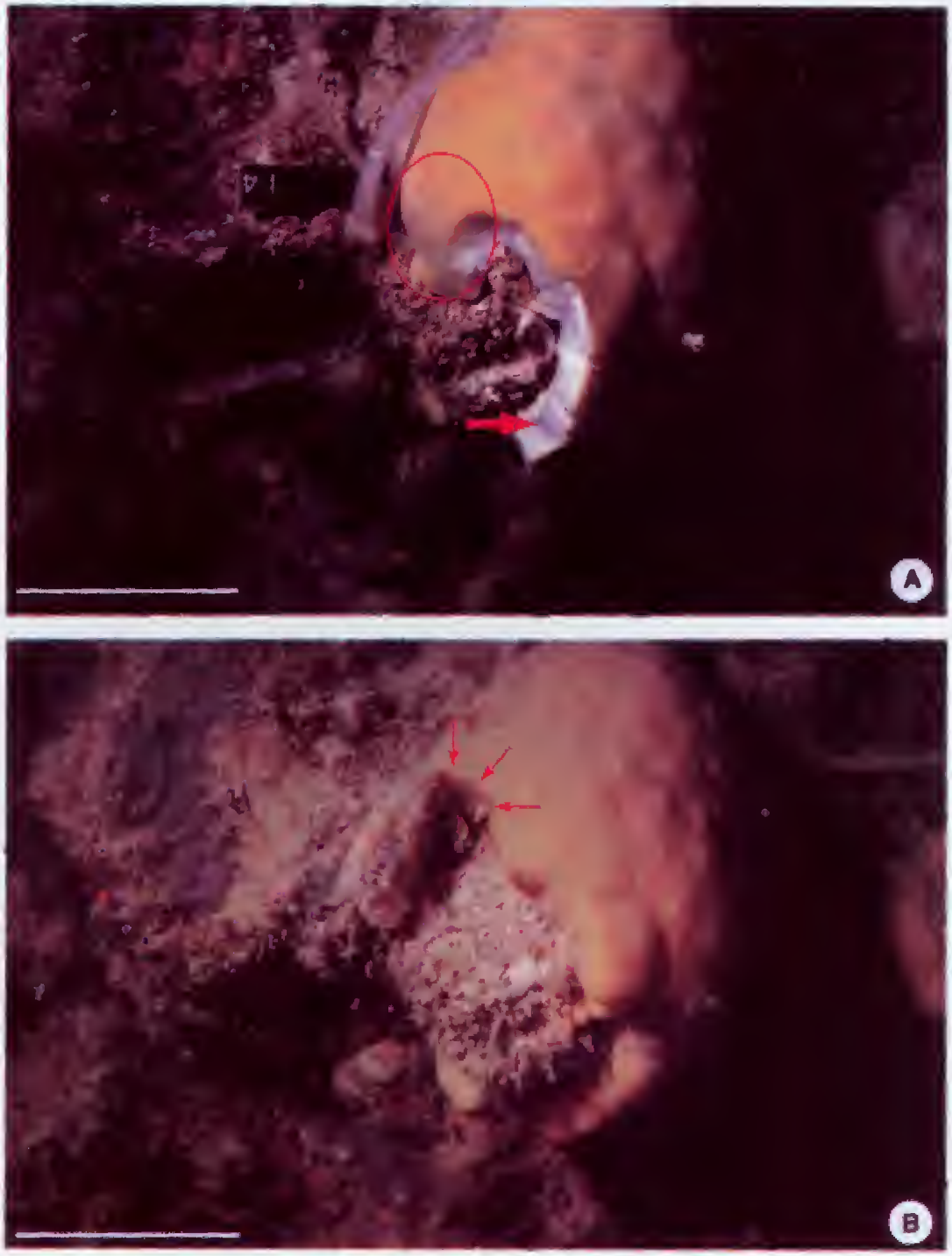

FIG. 8. *Ceratoporella nicholsoni*. A, Specimen no. 14 after a particularly severe sampling in May 1987. Arrow indicates fractured aragonitic skeleton. Encircled zone was found missing in 1997. (Scale bar=5cm); B, same specimen as in A, seen in May 1997. Zone fractured in 1987 has healed and shows round edges created by skeleton regeneration. Arrows indicate sharp edge of a more recent unexpected injury. (Scale bar=5cm).

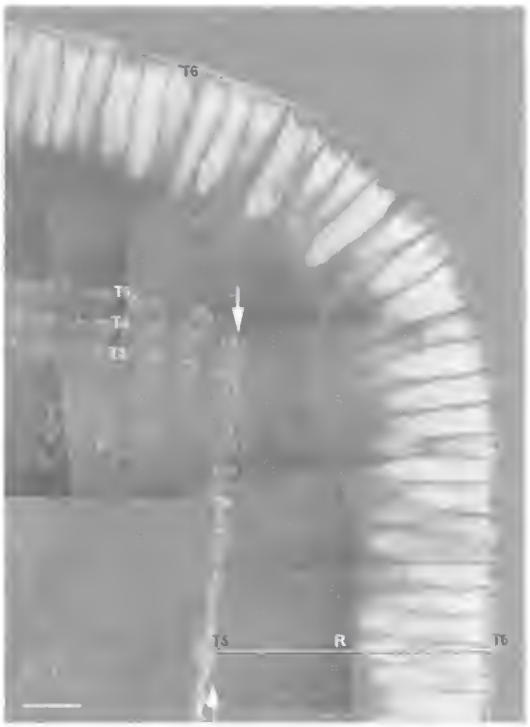

FIG. 9. *Ceratoporella nicholsoni*. Regeneration growth pattern. Ground section from specimen no. 10 sampled from its regeneration area in May 1997. Fracture, indicated by arrows occurred in May 1986, when a fragment was sampled. T6 indicates surface of skeleton marked with calcein 363 days later. R= regeneration growth axis. Epifluorescence microscopy. Captions as in Figure 1. (Scale bar= 500μm).

(Böhm et al., 1996)], or on direct methods (Dustan & Sacco, 1983; Willenz & Hartman, 1985). No details of analytical methods were reported by Dustan & Sacco (1983), who used alizarin red staining; their data remain approximate (0.1-0.2mm yr^{-1}). Initial values using calcein (Willenz & Hartman, 1985) were slightly lower (184.2±19.4μm yr^{-1}) than in the present study because measurements at the infilling zone at the base of pseudocalices were included in the latter study. Such artifacts were avoided here by rejecting measurements made in this zone, because they are too sensitive to minor deviations in the orientation of skeletal sections.

This study also provides clear evidence that statistically significant differences occur in the growth rate from one individual to another, within the same period of time. Moreover, the annual growth rate of a given individual also varied significantly with time.

Variations in growth rate were shown to occur in two other circumstances, suggesting that young tissues produce aragonite at a slower rate than tissues of older individuals. Firstly, measurements on a second population of smaller sized individuals, revealed a striking lower average annual growth rate (124.4±35.0μm yr^{-1}). Secondly, injuries made to large specimens induced horizontal regeneration zones which also appeared to reduce growth rate. In this latter case, however, after one year, growth was recorded but it was never higher than 18% of the normal growth figure. The impressive lateral regeneration growth rate (102-154 times faster) reported by Lehnert & Reitner (1997) corresponds to an ordinary extension of the living tissue produced to repair a damaged zone of the sponge prior to calcification. However, these authors did not record growth rates of the skeleton itself.

The present study examined populations of *Ceratoporella*, whereas most previous reports on growth rates were based on measurements of single specimens. These studies assumed a constant growth rate during the lifetime of the sponge. In this work we found that statistically significant variations are consequential, especially in researches relating growth rates and marine paleoenvironmental conditions such as water temperature or salinity, using skeletal chemistry of sclerosponges.

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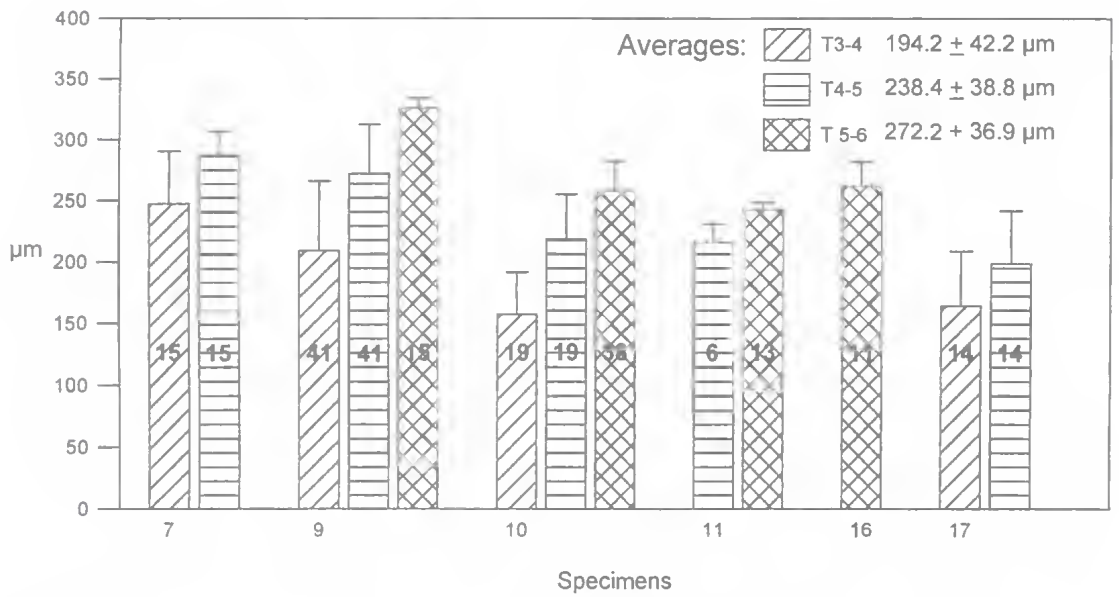

FIG. 10. *Ceratoporella nicholsoni.* Regenerative annual growth rate (µm yr^{-1}) of 6 specimens after injury caused by sampling. Conventions indicated as in Figure 3. A Kruskal-Wallis ANOVA on ranks and all pairwise multiple comparison test (Dunn's method) indicate a significant growth rate increment within a given specimen, from one period to the next one.

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